AD_____

Award Number: DAMD17-02-1-0500

TITLE: Genetic Influence on Toxicity and Prognosis in Women Treated with Breast Conserving Surgery and Radiation Therapy

PRINCIPAL INVESTIGATOR: Christine B. Ambrosone, Ph.D.
Jenny Chang-Claude, Ph.D.

CONTRACTING ORGANIZATION: Mount Sinai School of Medicine
New York, New York 10029-6574

REPORT DATE: August 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2004	Annual (8 Jul 03 - 7 Jul 04)

4. TITLE AND SUBTITLE
Genetic Influence on Toxicity and Prognosis in Women Treated with Breast Conserving Surgery and Radiation Therapy

5. FUNDING NUMBERS
DAMD17-02-1-0500

6. AUTHOR(S)
Christine B. Ambrosone, Ph.D.
Jenny Chang-Claude, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mount Sinai School of Medicine
New York, New York 10029-6574

E-Mail: Christine.Ambrosone@roswellpark.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumor and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. This variability results from common genetic polymorphisms. This study is conducted in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We are extracting DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced. Follow-up is ongoing, through clinic visits, letters, and home visits, and in the next year, we will correlate genotyping results with toxicity.

14. SUBJECT TERMS
Radiation sensitivity, DNA repair, oxidative DNA damage, genetic

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

INTRODUCTION: Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumor and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. Activity of many of the proteins involved in these processes are determined by common inborn genetic differences, termed genetic polymorphisms. We conducted a pilot study to determine if this were the case, and although the study population was mixed in stage at diagnosis and treatments received, we found that women with variant alleles that would allow more treatment-generated reactive intermediates to reach tumor cells had better survival.
We are conducting the present study in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We are extracting DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced.

BODY: Research accomplishments associated with each Task outlined in the Statement of Work will be addressed within the context of each of the objectives.

Technical Objective 1 Follow-up of breast cancer patients of the parent study regarding therapy outcome and survival and data collection.

The fieldwork, which could only commence as of April 2003 as reported earlier, is running smoothly and should be completed by the end of 2004.

Task 1: Months 1-2: Organization of recontact with patients through different sources, development of clinical data forms and questionnaire, and establishment of database.

Completed as reported in 2003.

Task 2: Months 3-24: Recruitment of patients through different sources, perform follow-up examination, obtain informed consent, collect clinical data, complete questionnaire

The personal data of the 478 patients were assembled from the four participating clinics in northern Baden-Württemberg for the follow-up. To achieve comparable follow-up interval between the follow-up examination and end of radiotherapy, the patients are divided into two groups for follow-up (table 1.)

	Group I	**Group II**
	Radiation in 1998/99	Radiation in 2000/01
Clinic	Number of patients	Number of patients
University of Heidelberg	145	124
St. Vincentius Hospital Karlsruhe	60	49
Municipal Hospital Karlsruhe	48	24

University of Mannheim	20	8
Total number of patients	273	205
Follow-up to be conducted in year	2003	2004
Interval since radiation	4 -5 years	3.5 -5 years

By the end of December 2003, we contacted 213 patients out of 273 in the first group. The documents of 210 patients are complete, for the rest of the patients the documents have to be completed either by requesting the patient or the attending physician. Ten patients are deceased and seven patients have refused participation. For those patients who refused further participation, Dr. Helmbold, a radio-oncologist who was responsible for the fieldwork, asked for information on their course of disease (regarding recurrence, metastasis and death) and for permission to use the previously collected blood sample for this study.

The local ethics committee of the participating Mannheim University Clinic demanded a separate written consent form including the heading of the Mannheim University Clinic. This requirement was fulfilled and the patients of the University clinic in Mannheim have also been contacted in the meantime

The follow-up of the patients of the second group commenced with the mailing of letters of invitation to study participation in January 2004. The remaining patients of the first group were combined with the second group, so that there are 265 patients to contact in 2004.

By the end of June, we have been able to contact 387 patients. Consent of full participation was achieved for 350 patients. 24 patients refused a follow-up examination but provided information on course pf disease; 16 also consented to use of their blood samples for genotyping. 13 patients have died in the meantime, from 9 of whom information on course of disease is available. 8 patients have refused permission to use the previously collected blood sample for this project.

Further procedures: month 25-29
All the remaining patients who have not made an appointment will be approached by telephone to arrange for a meeting either at home or in the clinic. Patients refusing to participate will be requested to provide information regarding their course of disease.
Patients will be asked to complete their documents.
Attending physicians will be contacted by mail or by telephone in order to obtain missing information.
Patients who have moved will be followed up through the registration offices to obtain their current addresses.
The validity of the information provided will be checked through clinical records, where possible.

Task 3: Months 24-36: Data entry with ongoing quality control and plausibility checks

In preparation for this task, we have developed a database for entry of the questionnaire data and clinical data on toxicity and outcome. Study data were entered on an ongoing basis. Data cleaning through plausibility control is performed at regular intervals. The datasets of 345 patients are already complete, whereas for 21 patients, data is missing for some items and must be collected.

Task 4: Months 30-36 Perform statistical data analysis; initial descriptive analyses, study of main effects of data derived from questionnaire.
To be completed in the third year.

Technical Objective 2 Evaluation of the effect of genetic polymorphisms in certain candidate genes (i.e. alleles that confer reduced protection from ROS damage and variants in DNA repair genes) and outcomes; i.e., breast cancer recurrence and severe skin toxicity.

Task 1: Months 3-6 DNA extraction and shipment of aliquot
Completed as reported in 2003.

Task 2: Months 26-30 Perform DNA analysis for genetic polymorphisms in genes that confer reduced protection from ROS damage, e.g. *MnSOD, GPX1, CAT, GSTT1, GSTM1, GSTA1, GSTP1*, and in DNA repair genes, associated with risk of cancer and/or ionizing radiation sensitivity e.g. *XRCC1, XRCC3, Ligase IV, XPD, APE1*
Genotyping is in process since we have experienced few refusals to using the previously collected blood sample. Genotyping has been completed for *MnSOD, GPX1, CAT, GSTT1, GSTM1, GSTA1, and GSTP1*. It is expected that the completion of genotyping will closely follow the completion of patient follow-up.

Task 3: Months 31-36 Merge data from laboratory results with questionnaire database. Perform statistical analysis for main effects of polymorphisms on outcomes.
Data analysis to assess the effect of the genetic polymorphisms on occurrence of acute toxicity, which was already observed in the parent study, will be carried out as soon as the genotyping is completed. Statistical analyses with respect to occurrence of prognosis will be completed in the second half of the third year.

KEY RESEARCH ACCOMPLISHMENTS:

- Assembled the personal data from 478 patients from four participating clinics in Germany. By the end of June 2004, we have been able to contact 387 patients. Consent of full participation was achieved for 350 patients.

- Developed a database for entry of the questionnaire data and clinical data on toxicity and outcome; study data entered and data cleaning performed at regular intervals. The datasets of 345 patients are already complete.

- Completed DNA extractions on all participating patients. Completed genotyping for several genes, assays are underway for remaining genes.

REPORTABLE OUTCOMES: None.

CONCLUSIONS: The recontacting and recruitment for participation of the patients has been extremely successful. We expect to achieve around 83 % full participation and to be able to

obtain information on clinical course without re-examination from another 8% and permission from all these patients to use the blood samples collected in the parent study for genotyping in this project. Genotyping is completed for several polymorphisms, and underway for others. We will begin data analysis for toxicity outcome during the coming year.

REFERENCES: None

APPENDICES: None